Lasers in Surgery and Medicine 22:207–211 (1998)

Quantitative Changes in Collagen Levels Following 830-nm Diode Laser Welding

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Background and Objective: **The actual mechanism by which laser irradiation welds tissue is presently unknown; however, collagen is a major constituent of tissue welded by laser irradiation.**
Study Design/Materials and Methods: **Collagen was extracted from the abdominal aorta of Wistar rats by acetic acid extraction and repeated pepsin digestion after tissue welding (254 W/cm^2) by using an 830-nm diode laser. The collagen levels were determined by using the Sircol Collagen Assay (Biocolor, Northern Ireland).**
Results: **Compared with untreated aorta, the collagen content of the treated vessel was obvious decreased ($P < 0.001$) immediately after laser irradiation. Levels then increased by day 3, with a peak at day 10 ($P < 0.002$). The collagen content returned to normal levels on day 30 and remained at this level throughout the rest of the experimental period.**
Conclusion: **These results suggest that a proportion of the collagen molecules in the vessel are denatured by the heat of the laser. Collagen synthesis is stimulated during the healing process after laser welding with the parameters used in the present study. Lasers Surg. Med. 22:207–211, 1998.** **© 1998 Wiley-Liss, Inc.**

Key words: diode laser; laser welding; collagen quantitative change

INTRODUCTION

Laser-assisted microarterial anastomosis (LAMA) is accomplished by directing a low-energy laser beam at the opposed edges of the vessel wall. This seals the tissue as effectively as suturing.

The mechanism of LAMA is presently unknown, but several studies have attempted to understand it. In 1986, Shober et al. [1] studied collagen from rat carotid arteries after Nd:YAG laser welding by electron microscopy. They demonstrated loosening of the collagen triple helix and some sort of interaction between collagen stands. They concluded that welding may occur by fusion of collagen fibers. White, Kopchok, and others [2–4] confirmed this fact with argon laser welding of vessels, but they proposed that collagen was not denatured after argon laser irradiation. However, other investigators [5,6] using the same laser type demonstrated that collagen denaturation had occurred. Brooks et al. [7], using a histological technique with picrosirius red F3BA staining, confirmed that vascular tissue was denatured in argon laser welding. Bass et al. [8] investigated the mechanism of diode laser welding with biochemical methods (SDS-polyacrylamide gel electrophoresis and circular dichroism) and also concluded that collagen molecules were denatured.

The aim of the present study was to investigate the quantity changes in native collagen molecules after laser welding and to evaluate the modifications in collagen levels during the healing process after 830 nm diode LAMA.

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Accepted 13 January 1998

MATERIALS AND METHODS

Sixty-four Wistar rats ranging in weight from 350 to 500 g were used in the present study. The animals were randomly assigned from a computer-generated assignment table and divided into seven groups representing a control group (n = 16) and six laser-treated groups taken at various postoperative times: 0 (immediately after laser irradiation), 3, 10, 30, 60, and 120 days (each group n = 8). Animals were anesthetized by intramuscular injection of a mixture Vetranquil® (0.4 mg/kg body weight) and Ketamine (80 mg/kg body weight). Laparotomy was performed, and all operative procedures were carried out with standard microsurgical techniques under an operating microscope (6–25× magnification, Zeiss OPMI 1, Berkochen, Germany). The aorta was liberated from the posterior plane by section of the illiolumbar and testicular arteries after electrocoagulation and mobilized from the renal vessels to the aortic bifurcation. This part of abdominal aorta was used in the study. The blood flow was occluded with two microclamps, and the vessel lumen was irrigated with 0.9% saline solution through a small opening until the color of the aortic wall was white. The water was then wiped away with a compress, and the vessel was nearly dry at the time of laser application. After laser treatment, the small opening was closed by laser welding or by suture (one or two stitches), if necessary. No anticoagulant was used.

Laser Treatment

The diode laser, providing a continuous wave laser beam of 830 nm, was a prototype from the Swiss Federal Institute of Technology (Lausanne, Switzerland). The laser beam was focused into an optic fiber delivery system (300 μm) and transmitted to the vessel surface through a micromanipulator (a prototype developed in our laboratory) coupled to the operating microscope, giving a spot size of 1 mm and guided by a diode red light (670 nm). Laser treatment considered of regular arterial wall irradiation using juxtaposed shots. Each shot had a power of 2 W and was 8 seconds in duration, thus giving a dose of 254 W/cm^2. This condition followed our previously reported parameters for diode LAMA [9]. The anterior vessel face was treated first and the posterior one afterward. Between 48 and 60 shots were applied to each aorta. The total dose of energy was 897 ± 52 J.

Collagen Extraction and Measurement

The laser-treated and untreated abdominal aortas were harvested from the infrarenal vessels to the aortic bifurcation. The specimens were washed with physiological saline, minced with scissors, and then homogenized in ethanol with an ultrahomogenizer (Ultra-turrax, Model T25, Stanfon, Germany). The delipidized dry weight (DDW) was determined after drying with a speed vac concentrator (Savant, Hicksville, NY). Repeated pepsin digestion was done by using a modification of the method of Murata et al. [10]. The sample was immersed in 0.5 M acetic acid for 48 hours and then digested with pepsin (Sigma, Paris, France) at 100 mg/g DDW, with constant stirring for 24 hours. Further enzymatic treatment was carried out twice with the addition of pepsin at 50 mg/g DDW for 24 hours each time. After centrifugation at 18,000*g* for 1 hour, the pellet was redigested with pepsin once more and centrifuged. The two supernatants were pooled and dialyzed against 2 mM Na_2HPO_4 at 4°C to precipitate the collagen. The precipitates were dissolved in 0.5 M acetic acid and centrifuged to remove any insoluble material. The collagen contents of the supernatants were determined with the Sircol Collagen Assay (Biocolor, Northern Ireland) as described by the manufacturers.

Statistical Analysis

Statistical differences between groups were assessed by analysis of variance and testing for significance with the unpaired Student's *t*-test. Data are expressed as mean ± 1 standard deviation, and mean values were considered different if *P* values were less than 0.05.

RESULTS

Clinical Findings

Immediately after laser treatment, the vessel wall showed a slight whitish discoloration and was slightly transparent (Fig. 1a). This color was similar to that seen at the anastomotic site after diode laser-assisted welding.

From day 10, there was obvious and regular luminal dilatation of all abdominal aorta that had been treated with the laser. The diameter of the vessel was almost double that before laser treatment and of the control group (Fig. 1b). It varied until day 120.

There was no mortality in any group.

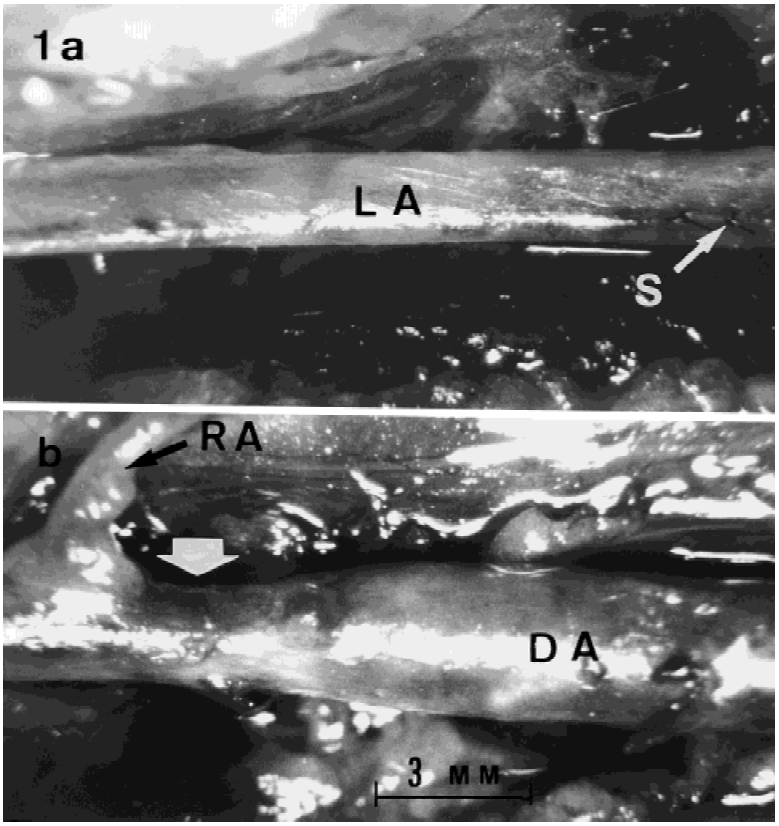

Fig. 1. Effect of 830-nm diode laser irradiation on rat aorta. **a:** Immediately after laser irradiation, the vessel wall shows a slight whitish discoloration and is slightly transparent. LA, laser treated abdominal aorta; S, stitches for closing the opening. **b:** On day 60 after laser irradiation, the lumen of all of abdominal aorta is obviously and regularly dilated. The diameter of the vessel is almost double that before laser treatment. RA, right renal artery; DA, dilated abdominal aorta. The white arrow shows the site where the vessel was clamped during laser treatment.

Delipidized Dry Weight

The mean DDW of the vessel wall is shown in Table 1 and depicted graphically in Figure 2. The DDW did not decrease immediately after laser irradiation. However, by day 10 it had increased when compared with nonirradiated controls ($P < 0.05$). This effect was transient and the DDW decreased rapidly, returning to normal levels by day 30, and remained at this level throughout the rest of the experimental period.

Collagen Content

The change in collagen levels is shown in Table 1 and depicted graphically in Figure 3. Immediately after laser irradiation, the collagen content of the treated vessel wall was reduced ($P < 0.001$). Following an initial increase on day 3, there was a peak on day 10. It was significantly higher than that of the control group ($P < 0.002$). The collagen content returned normal levels on day 30 and remained normal until day 120.

TABLE 1. Changes (Mean ± SD) in Delipidized Dry Weight (DDW) and Collagen Level After 830 nm Diode Laser Welding

Time	DDW (mg)	Collagen content (μg)
Control	5.6 ± 0.9	549.3 ± 91
D0	5.5 ± 0.7	313.1 ± 84*
D3	5.1 ± 1.0	474.1 ± 94
D10	8.9 ± 1.9**	794.6 ± 177***
D30	6.8 ± 1.1	528.3 ± 110
D60	7.4 ± 2.1	527.3 ± 120
D120	6.8 ± 1.9	488.8 ± 66

*$P < 0.05$
**$P < 0.001$
***$P < 0.002$

DISCUSSION

The proportions of different collagen types in vessel walls have been established. Type I collagen constitutes 60%, type III constitutes 30%, and the remaining 10% is constituted by type V and minor collagens [11]. Acetic acid extraction does not denature the protein molecules and pepsin

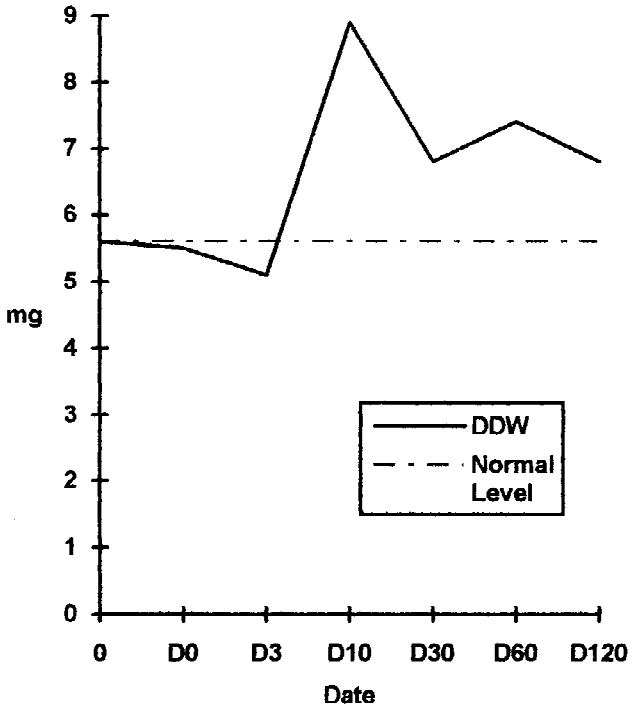

Fig. 2. Changes in delipidized dry weight (DDW) after 830 nm diode laser welding. The DDW decreases immediately after laser irradiation and increases significantly on day 10. It returns to normal levels by day 30 and remains at this level throughout the rest of the experimental period.

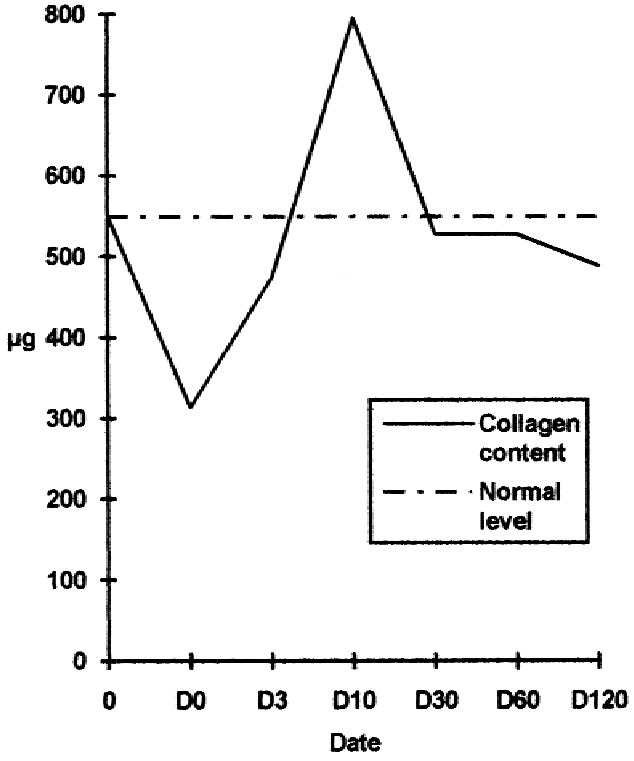

Fig. 3. Changes in collagen levels after 830 nm diode laser welding. Immediately after laser irradiation (D0), the collagen content of the treated vessel wall is reduced significantly. Following an initial increase on day 3, there is a peak on day 10, and it is significantly higher than the peak for the control group. The collagen content returns normal levels on day 30 and remains normal until day 120.

cleaves only nonhelical collagen, leaving helical collagen molecules intact [8].

The Sircol collagen assay is a quantitative dye-binding method designed for in vitro analysis of collagens. It can measure mammalian collagen types I–V. This reagent contains sirius red, which is an anionic dye with sulphonic acid side chain groups. These groups react with the side chain groups of the basic amino acids present in collagen. The specific affinity of the dye for collagen under the assay conditions is due to the elongated dye molecules becoming aligned in parallel with the long, rigid helical structure of native collagens (technical information of Sircol collagen assay).

The mechanism of tissue welding by diode laser probably involves a part of collagen denaturation. We have observed a decrease in the amount of collagen after laser irradiation with the condition following our previous report for LAMA [9]. The denatured collagen is cleaved by pepsin when extracted [8]. An obvious arterial luminal dilatation after diode laser treatment also suggests that collagen structure is damaged by laser heating and that vessel lumen is dilated by blood pressure. However, the vessel wall is not thickened because the weight of the wall (DDW) did not increase after day 30. We have also noted that a part of collagen is retained after laser irradiation. The remaining part of the helix structure of collagen could be explained by the fact that the fibrils of collagen unraveled under laser heat and re-entwined during the cooling phase [12,13].

Collagen synthesis is observed in laser-welded vessels from day 3 to day 10 of the postoperative period. A stimulation of collagen production by laser irradiation was also observed by Abergel et al. [14] in cultured cells. White et al. [15] measured the incorporation of radioactive hydroxyproline into collagen and found that the rate of collagen synthesis in the Nd:YAG laser-assisted venotomies at 1, 4, and 5 weeks was approximately twice that of sutured anastomosis. We observed only one peak of collagen synthesis, at day 10, but our methodology was not as sensitive as that used by White et al. The collagen content returned to normal levels at day 30 and remained normal for the rest of the experimental

period. Our previous histological findings [16] suggest that collagen synthesis plays an important role in healing during the first few days after irradiation; from day 3 to day 10 after diode LAMA, there is a period of re-endothelialization, with an inflammatory infiltration at the adventitia. After day 30, there is a reconstruction of the elastic laminae and a retructuration of the media.

There are several types of collagen in the vessel wall, and each type contains different proportions of hydroxyprolin residues, which stabilize the helical conformation of collagen molecules and result in greater temperature stability [17]. Further studies are needed to determine the effects of after diode laser welding on each collagen type and to determine the proportion of different types of collagen during the healing process.

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